I. STRUCTURAL STUDIES OF METAL PROTEIN COMPLEXES II. A CRITICAL STUDY OF THE PFEIFFER PHENOMENON III. NUCLEOPROTEIN AND THE ELECTRON MICROSCOPE

BY

MARY LOUISE QUAIFE

A. B., University of Michigan, 1938
M. S., University of Michigan, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1942

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ACKNOWLEDGMENT

Appreciation is expressed for the able counsel which Professor George L. Clark has given in this study. The cooperation and guidance of Dr. Martha Barnes Baylor for the section dealing with nucleoprotein were likewise of much assistance.

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INTRODUCTION

Classical chemical methods for structural study of protein and protein-like materials have been augmented recently by the use of instruments.

In this study the electron microscope revealed protein morphology in finer detail than could be shown previously. X-ray diffraction methods were used qualitatively to demonstrate identity or non-identity of crystal structure, varying degree of complexity of the crystal lattice and degree of crystallinity. Spectrophotometric study was used to compare structure on the basis of modern color theory which holds that an absorption band may be due to oscillation within a particular molecular grouping provided that part contains an excellent resonance system within itself.

EXPERIMENTAL AND RESULTS

I. The Hardy automatically recording spectrophotometer was used to determine absorption in the visible range (400 millimicrons to 700 millicrons) for the copper compounds of asparagine and picolinic acid, the copper biuret compounds of malonamide and glycinamide, and the biuret reaction mixtures of gelatine and crystalline egg albumin.

Spectra of the latter four are very similar but unlike either of the first two. Maximum and minimum of transmission for the biuret compound of malonamide and the biuret reaction mixture of egg albumin are identical, 435 millimicrons and 552 millimicrons, respectively. Spectra of the first four copper complexes are not complicated by excess cupric ions as in the case of the usual biuret reaction.

X-ray diffraction powder patterns of the copper complexes show markedly different crystal structure from that of the

uncombined amine compounds. They seem to be members of simpler crystal systems. It was easier to obtain smooth powder interferences with the copper complexes than with the amine compounds which were more difficult to grind.

II. A critical study of the Pfeiffer test was made. Pfeiffer modified the macro crystallization pattern of cupric chloride by the addition of minute traces of protein-containing substances. He found a characteristic pattern when cancerous blood was used, groups of crosses, unlike the normal blood pattern, radiation of crystals from a common center.

Modified Pfeiffer technique confirmed the following: Under controlled environmental conditions highly characteristic patterns of cupric chloride with glycogen or with starch are given. Protein addition agents give a different pattern than these two, though the effect of various proteins is the same. Addition of inorganic salts produces no effect. Cupric chloride alone and cupric chloride crystallized with another salt as impurity show only scattered patches of heaped-up crystals.

X-ray diffraction powder patterns of samples from these plates of cupric chloride alone and of cupric chloride with gelatine, starch, or glycogen show that crystallization of cupric chloride with the impurity gives a crystal lattice identical to that of the pure salt. Only the crystal habit is changed.

Pfeiffer tests with blood from three cases of advanced cancer were negative. These patients had received X-ray therapy, a previously suggested cause of negative results. Blood from both cancerous mice and normal mice sometimes gave positive and sometimes negative findings.

Duplicate tests run on the same specimen sometimes failed to check, even though the prescribed precautions of previous investigators were maintained carefully. This is ascribed to etching of the glass plates by repeated contact with cupric chloride solution.

Other salts which were substituted for cupric chloride, notably cobalt chloride and sodium chloride, exhibit the Pfeiffer effect. Crystal patterns are of course different in each case.

III. Morphology and fibrous character of nuclear material were studied. Use was made of the electron microscope, X-ray diffraction, and Pfeiffer macro crystal patterns.

An extension of the Pfeiffer effect was observed in comparing electron micrographs of chromosomes dried from salt solution to those from which salt crystals had been removed by washing. They show a different morphology when crystallized from aqueous medium than crystallized from salt solution.

Nucleoprotein was prepared from five natural sources. It gives a beautiful fern-like pattern when crystallized in the presence of salt. Egg albumin with salt exhibits the same pattern under the electron microscope. Macro crystal patterns of sodium chloride with a trace of egg albumin added show this pattern under the optical microscope.

The electron microscopic pattern is not shown by sodium chloride with protamine, peptone, or glycogen. Salt plus synthetic protamine thymonucleate or lysine yeast nucleate has similar but not identical appearance.

X-ray powder diffraction patterns of structural components of nucleoprotein were made. Patterns of protamine, thymonucleic acid, yeast nucleic acid and its zinc and copper salts, arginine yeast nucleate, and lysine yeast nucleate show either a single diffuse halo or a uniform blackening over the exposed area. Apparently in the powder state they are chiefly amorphous.

Fibers of nucleoprotein were formed readily by dilution of its sodium chloride solution with water. An extensive network of fibrils shows clearly in an electron micrograph. However, no preferred orientation could be found in x-ray diffraction patterns of fibers stretched over two hundred per cent and dried thus.

SUMMARY AND DISCUSSION

I. The very similar absorption curves of biuret compounds of malonamide and glycinamide to those of the biuret reaction mixtures of egg albumin and gelatine suggest that that por-

tion of each molecule which coordinates with the copper and gives rise to the color must have similar resonance characteristics and related structure.

II. The presence of impurity in the crystallizing cupric chloride (Pfeiffer effect) does not change its plan of internal structure as shown by X-ray examination. The external appearance of the crystal groups is changed characteristically by certain agents, notably polysaccharides. It may be explained as due to adsorption of impurities on growing crystal faces and a consequent change in growth rate of crystals in different directions. Since the effect is not always reproducible even under rigidly controlled conditions, it cannot be utilized as a conclusive diagnostic test for cancer.

III. A delicate fern-like pattern of crystals has been found for salt-protein mixtures when nucleoprotein, from any of five natural sources, or egg albumin is the protein component. Certain other nucleates and simpler protein substances cannot serve as the protein component. Production of this pattern may depend upon possession of fibrous character and of a certain minimal molecular weight by the protein portion.

VITA

The author was born on December 8, 1917, at Madison, Wisconsin. She attended the public schools of Highland Park, Michigan, including Junior College.

From the University of Michigan, she received a B.A. in 1938 and a M.S. in biological chemistry in 1939.

She had charge of the blood chemistry laboratory at Vanderbilt University Hospital for the year 1939-40.

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